

What is claimed:

1. A method for purifying highly anionic target proteins in a sample from a plurality of proteinaceous and non-proteinaceous impurities and DNA/histone complexes in the sample comprising contacting the sample with a substrate capable of reversibly binding charged molecules under conditions appropriate for binding of the anionic target proteins to the substrate, and purifying the highly anionic target proteins under conditions such that the plurality of proteinaceous and non-proteinaceous impurities in the sample either do not bind or are washed off the substrate while the highly anionic target proteins remain bound, and such that the DNA/histone complexes in the sample are dissociated, thereby purifying highly anionic target proteins in the sample.

2. The method of claim 1, wherein the target protein is a sulfated protein, and the impurities include a hyposulfated form of the target protein.

3. The method of claim 2, wherein the sulfated protein is P-Selectin Glycoprotein Ligand-1 or P-Selectin Glycoprotein Ligand-1 fusion protein.

4. A method for purifying highly anionic target proteins in a sample from a plurality of proteinaceous and non-proteinaceous impurities and DNA/histone complexes in the sample comprising (a) contacting the sample with a substrate capable of reversibly binding charged molecules whereby the target proteins bind to the substrate, (b) washing the substrate with a first wash solution under appropriate conditions whereby the plurality of proteinaceous and non-proteinaceous impurities either do not bind or are washed off the substrate while the target proteins remain bound, (c) eluting the sample with a first elution solution wherein the first elution solution comprises a salt solution at a high molar concentration, and (d) collecting the eluted sample containing the purified anionic target proteins.

5. The method of claim 4, wherein the pH of the first wash solution is about 4.0 to 8.0.

6. The method of claim 4, wherein the pH of the first wash solution is about 6.5.

7. The method of claim 4, wherein the sample which is eluted from the substrate is further purified.

8. The method of claim 7, wherein the further purification comprises (a) passing the eluted sample containing the target proteins through a metal chelate chromatography column or a hydrophobic interaction chromatography column whereby the eluted sample is captured on the column, (b) washing the column with a second wash solution under appropriate conditions whereby DNA/histone complexes contained in the sample are dissociated, (c) eluting the sample with a second elution solution, and (d) collecting the eluted sample containing the purified anionic target proteins.

9. The method of claim 8, wherein the second wash solution comprises a high salt concentration and the second elution solution comprises a lower salt concentration than the second wash solution.

10. The method of claim 8, wherein the eluted sample containing the target proteins is passed through a hydrophobic interaction chromatography column, the second wash solution selected from the group consisting of NaCl with a concentration of about 4M and ammonium sulfate with a concentration of about 1.2M, and the second elution solution is ammonium sulfate with a concentration of about 0.48M.

11. The method of claim 9, wherein the second wash solution is selected from the group consisting of (a) a solution comprising NaCl at about 4M and Tris at about 20mM and a pH of about 7.4, (b) a solution comprising isopropanol at about 5% and ammonium sulfate at about 1.2M, (c) a solution of ethanol at about 5% and ammonium sulfate at about 1.2M, and (d) a solution of ethanol of about 5% and NaCl at about 4M.

12. The method of claim 9, wherein the eluted sample containing the target proteins is passed through a metal chelate chromatography column, the second wash solution comprises a salt concentration of about 2M, and the second elution solution comprises a salt concentration of about 200mM to 1M.

13. The method of claim 12, wherein the second elution solution comprises a salt concentration of about 1M.

14. The method of claim 12, wherein the second wash solution comprises MES at about 40mM, NaCl at about 2M, and imidazole at about 5mM, and the second elution solution comprises a solution of MES at about 40mM, NaCl at about 1M, and imidazole at about 35mM.

15. The method of claim 4, wherein the target proteins have at least about one (1) sulfate group.

16. The method of claim 5, wherein the target proteins have at least about five
5 (5) sulfations.

17. The method of claim 4, wherein the proteins are P-Selectin Glycoprotein Ligand-1 or P-Selectin Glycoprotein Ligand-1 fusion proteins.

10 18. The method of claim 4, wherein the proteins are recombinant proteins.

19. A method for purifying highly anionic target molecules comprising an immunoglobulin Fc domain in a sample from a plurality of proteinaceous and non-proteinaceous impurities and DNA/histone complexes in the sample comprising contacting
15 the sample with a substrate capable of binding the Fc portion of the highly anionic target molecules under conditions such that the plurality of proteinaceous and non-proteinaceous impurities either do not bind or are washed off the substrate while the target proteins remain bound, and such that DNA/histone complexes in the sample are dissociated, thereby purifying highly anionic target molecules from DNA/histone complexes in the sample.

20 20. The method of claim 19, wherein the target molecule is a sulfated protein, and the impurities include a hyposulfated form of the target molecule.

21. The method of claim 19, wherein the sulfated molecules are P-Selectin
25 Glycoprotein Ligand-1 fusion proteins.

22. The method of claim 21, wherein the P-Selectin Glycoprotein Ligand-1 fusion proteins are rPSGL-Ig.

30 23. The method of claim 19, wherein the non-proteinaceous and proteinaceous impurities are washed off the column with NaCl having a concentration of about 1M.

24. The method of claim 19, wherein the non-proteinaceous and proteinaceous impurities are washed off the column with NaCl having a concentration of about 1M and
35 wherein the highly anionic target molecules are P-Selectin Glycoprotein Ligand-1 fusion proteins.

25. The method of claim 24, wherein the P-Selectin Glycoprotein Ligand-1 fusion proteins are rPSGL-Ig.

26. A method for purifying highly anionic target molecules comprising an immunoglobulin Fc domain in a sample from a plurality of proteinaceous and non-proteinaceous impurities and DNA/histone complexes in the sample comprising (a) contacting the sample with a substrate capable of binding the Fc portion of the highly anionic target molecules whereby the target immunoglobulins bind to the substrate, (b) washing the substrate with a first wash solution under appropriate conditions whereby the plurality of proteinaceous and non-proteinaceous impurities in the sample either do not bind or are washed off the substrate while the target molecules remain bound, (c) eluting the sample with a first elution solution wherein the pH of the first elution solution is low, and (d) collecting the eluted sample containing the purified highly anionic target molecules.

27. The method of claim 26, wherein the pH of the first elution solution is about 4.0.

28. The method of claim 26, wherein the eluted sample containing the purified anionic target molecules is further purified.

29. The method of claim 28, wherein the further purification comprises the (a) contacting the eluted sample containing the purified anionic target molecules with a second substrate capable of reversibly binding charged molecules whereby the target immunoglobulins bind to the second substrate, (b) washing the second substrate with a second wash solution wherein the pH of the second wash solution is low thereby removing the proteinaceous and non-proteinaceous impurities in the sample, (c) eluting the sample with a second elution solution, and (d) collecting the eluted sample containing the purified anionic target molecules.

30. The method of claim 29, wherein the proteinaceous impurities consist in part of leached Protein A.

31. The method of claim 29, wherein the pH of the second wash solution is about 4.0.

32. The method of claim 26, wherein the target molecules have at least about one (1) sulfation(s).

33. The method of claim 26, wherein the target molecules have at least about five (5) sulfations.

34. The method of claim 26, wherein the molecules are P-Selectin Glycoprotein
5 Ligand-1 fusion proteins.

35. The method of claim 34, wherein the P-Selectin Glycoprotein Ligand-1 fusion proteins are rPSGL-Ig.